Gram-Negative Cocci

Introduction

The OME version of bacterial taxonomy lessens the burden of memorization in exchange for simplicity. Technically, there are Gram-negative diplococci (*Neisseria* and *Moraxella*) and Gram-negative coccobacilli (*Haemophilus*, *Bordetella*, *Pasteurella*, *Brucella*, and *Francisella*). Coccobacillus means somewhere in-between rods and cocci. For a bacteriologist, these are important distinctions. To a clinician, the diseases they cause are far more relevant than the details about their shape or their technical taxonomy. So, we cheat.

Pasteurella, *Brucella*, and *Francisella* are sent to their own lesson, the zoonotic bacteria. Pertussis is discussed in Immunology. That leaves the three diplococci and one almost-a-coccus to be combined into just "cocci." What we are left with is the Gram-negative cocci. There are only four organisms to learn: *Neisseria meningitidis* causes meningitis, *Neisseria gonorrhoeae* causes gonorrhea, and "the other *Strep. pneumo*" bacteria pair—*Haemophilus influenzae* and *Moraxella catarrhalis*—cause otitis media, sinusitis, and pneumonia (like *Strep. pneumo*). In the Gram-positive cocci lesson, we showed you how to harness similarities to make studying those bacteria easier. In this lesson we flip it. Do not be tricked by genus, do not cluster; learn them as distinct, separate organisms. *Neisseria gonorrhoeae* has more in common structurally with *Moraxella catarrhalis* than it does with *Neisseria meningitidis*. The disease *Moraxella catarrhalis* causes has more in common with *Haemophilus influenzae* than with the bacteria it most structurally resembles, *Neisseria gonorrhoeae*. When you learn Gram-negative cocci, say both the genus and the species every time, never abbreviating the organism. We will refer to *Neisseria meningitidis* as *Neisseria meningitis* (except in tables)—it is easier to say, and it keeps you focused on the disease it causes.

Laboratory Diagnostics

As we mentioned in Bacteria #4: *Laboratory Diagnosis*, the Gram-negative tests are maltose fermentation, lactose fermentation, oxidase test, and H_2S production. For Gram-negative cocci the only test we'll need is **maltose fermentation.** The only organism that is a maltose fermenter is *Neisseria meningitis*.

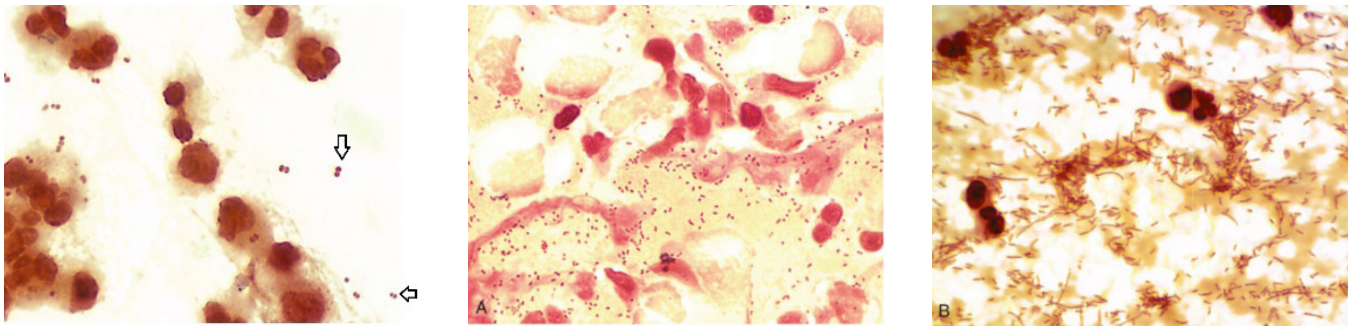
Agars are implemented heavily here. Every one of the organisms in this lesson, except one, needs to be associated with a specific agar, as shown in Table 7.1. Remember that Thayer-Martin agar is chocolate agar with antibiotics. That means *Neisseria* can grow on chocolate agar, but is allowed to flourish on Thayer-Martin. *Haemophilus influenzae* can only grow on chocolate agar with X factor (hemin) and V factor (nicotinamide adenine dinucleotide, NAD, "vitamin" factor). It cannot grow on chocolate agar alone, nor can it grow on Thayer-Martin agar.

ORGANISM	DIAGNOSTIC	DISEASE IT CAUSES	CAUTION
Neisseria meningitidis	Thayer-Martin agar, and DOES ferment maltose	Rapidly progressive meningitis	Can grow on chocolate agar without factors
Neisseria gonorrhoeae	Thayer-Martin agar, and does NOT ferment maltose	Gonorrhea	
Bordetella pertussis	Bordet-Gengou agar	Pertussis (whooping cough)	Not in this lesson
Haemophilus influenzae	Chocolate agar with factors X and V	Pneumonia	
Moraxella catarrhalis	Not any of the others	Pneumonia	

Table 7.1: Gram-Negative Cocci Diagnoses and Diseases by Organism

Gram Stain

Since we're cheating and calling all of these cocci, it does seem appropriate at least to show you what these things would look like if we hadn't cheated. *Neisseria meningitis*, *Neisseria gonorrhoeae*, and *Moraxella catarrhalis* are **diplococci**, found in pairs. *Haemophilus influenzae* and *Bordetella pertussis* are **coccobacilli**, which means they can be rod-shaped (pleomorphic) or coccus-shaped.



(a) (b) (c)

Figure 7.1: Gram Stains of Gram-Negative "Cocci"
(a) Diplococci from a sputum sample (arrows). (b) Small coccobacillus forms seen in sputum from patient with pneumonia. (c) Thin pleomorphic forms seen in a one-year-old unvaccinated child in Africa with overwhelming meningitis.

Neisseria meningitidis ("Causes Meningitis," aka "Meningococcus")

Neisseria meningitis (our term) is the Neisseria species that causes the rapid onset, quickly fatal meningitis. It is the meningitis that takes a healthy teenager and within 24 hours kills them. It is associated with a diffuse petechial rash, can cause septic shock, adrenal insufficiency, and disseminated intravascular coagulation (Waterhouse-Friderichsen syndrome), and is entirely preventable with a vaccine.

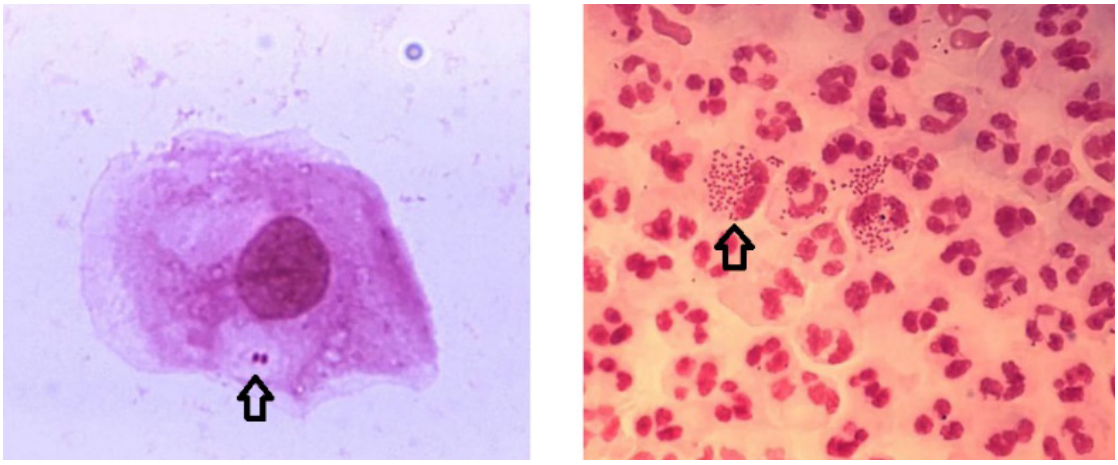
Physiology/Structure. *Neisseria meningitis* is Gram-negative kidney-bean-shaped **diplococci** with flattened sides. *Neisseria meningitis* is a **facultative intracellular** organism; therefore it is small in size and colonies will not regularly grow on regular blood agar. The **Thayer-Martin** agar is chocolate agar with some antibiotics to kill off competing colonies, killing everything except *Neisseria*. *Neisseria* can grow on chocolate agar alone and without factors (caution: *Haemophilus influenzae* grows on chocolate agar but needs factors X and V), but Thayer-Martin ensures that only *Neisseria* grows.

Virulence. *Neisseria meningitis* has a polysaccharide **capsule.** There are 5 different serotypes and subtype B is the most common in the US. This capsule is large, allowing for rapid detection and diagnosis of the CSF with **latex particle agglutination**. This capsule also allows for a vaccine to be developed. **IgA protease** allows colonization of the respiratory and oropharyngeal epithelium by degrading the mucosal antibody. **Pili** are used for attachment to the epithelium and invasion. Patients infected by this organism become worse through an effect of the immune system responding to the presence of the bacteria, not as a result of tissue damage done by the bacteria. The outer plasma membrane of *Neisseria meningitis* does **not have any lipo-poly-saccharide**, but instead has an even more antigenic **lipo-oligo-saccharide**. The robust antigenic response accounts for its rapid and catastrophic presentation.

Epidemiology. *Neisseria meningitis* is found in the human nasopharynx, with about 10–15% of humans as asymptomatic carriers. It is transmitted from person to person through **respiratory droplets**. The bacteria colonize the nasopharynx. Colonization rarely results in disease. Disease occurs when the bacteria reach the brain via the bloodstream. Because of its catastrophic presentation and ease of transmission through respiratory droplets, those at highest risk are those who are confined to close quarters—**college dorm residents**, **military recruits**, and those in refugee camps.

Disease. Meningitis caused by *Neisseria meningitis* takes a **precipitous course**. The patient goes from normal to dead in 24 hours. The disease begins with the typical meningeal signs—**stiff neck, photophobia,** and **fever**. Once a **petechial rash** forms, the patient declines quickly. The petechial rash plus meningitis is the tip-off for meningococcal meningitis. Other causes of meningitis are not so abrupt and do not have the rash. The lipo-oligo-saccharide causes profound inflammation, resulting in septic shock. The inflammation promotes **disseminated intravascular coagulation**. The bacteria themselves do not penetrate or infect the adrenal glands, but meningitis with *Neisseria meningitis* can cause **adrenal failure** secondary to hypotension and DIC, leading to a hemorrhagic adrenalitis called Waterhouse-Friderichsen syndrome.

Treatment. Because the diagnosis is "meningitis" until bacterial cultures come back, empiric coverage is for all the bugs that can cause meningitis—use ceftriaxone, vancomycin, and steroids. The agglutination test can be performed on CSF, and the vancomycin de-escalated if positive. Any close contacts should receive the vaccine and **prophylaxis with rifampin**.



(a) (b)

Figure 7.2: Intracellular Organisms
(a) *Neisseria meningitidis*, the diplococcus indicated by the arrow, can be seen within this cell taken from the CSF of a patient with meningitis. (b) A low-powered view shows dozens of neutrophils, some laden with small dots. Those dots are intracellular *Neisseria meningitidis*.

Neisseria gonorrhoeae ("Causes Gonorrhea")

Neisseria gonorrhoeae is the infectious organism that causes the disease gonorrhea. The disease gonorrhea has many differing presentations, discussed below. We are calling out this distinction multiple times to burn it into your brain that *Neisseria gonorrhoeae* and *Neisseria meningitis* have a name that is similar to one another and they look the same—diplococci grown on Thayer-Martin agar. The similarities end there.

Physiology/Structure. *Neisseria gonorrhoeae* is Gram-negative kidney-bean shaped **diplococci** with flattened sides. *Neisseria gonorrhoeae* can be grown only on **Thayer-Martin medium** (chocolate + antibiotics) and will not grow on blood agar. It is a facultative intracellular organism; therefore it is small, and organisms are rarely visible on Gram stain. It ferments only glucose, and because it is not *Neisseria meningitis*, it **does not ferment maltose**.

Virulence. *Neisseria gonorrhoeae* gets into mucosal epithelium, gets into the cells themselves, and replicates. To do that it uses pili, porins, and opa proteins. The **pili** are inner membrane proteins that penetrate through the peptidoglycan cell wall and the outer membrane. Pili allow tight adhesion to nonciliated mucosal cells (mouth, vagina, anus). **Porins** are outer membrane proteins that allow the cell

to ingest nutrients and expel waste—while inside another cell. **Opa** (opacity) proteins facilitate adhesion and cell-to-cell communication. There is **no capsule** on *Neisseria gonorrhoeae*; the bacteria rely on **antigenic variation** to avoid detection by the immune system.

Epidemiology. Gonorrhea is a sexually transmitted infection. It lives in mucosal epithelium. Wherever semen can go, gonorrhea can go (pharynx, anus, vagina). Whatever epithelium is in the vagina can acquire gonorrhea (sex and childbirth) from the vagina.

Male urogenital gonorrhea presents most often as **urethritis** (purulent yellow discharge from the penis with painful urination). Very few cases are asymptomatic. And since unintentional discharge from a penis is usually noticed, men usually seek medical care.

Female urogenital gonorrhea is the inverse; it is usually asymptomatic in women. When symptomatic, the presentation can be as minor as **cervicitis** (painful sex, mucopurulent discharge from the cervix, tender cervix) to **acute pelvic inflammatory disease**. Untreated infections can cause sterility. Bacteria can ascend the fallopian tubes and enter the abdominal cavity, causing perihepatic infection (**Fitz-Hugh–Curtis** syndrome). Gonorrhea may go unnoticed in women and so they become the common carrier. These concepts are considered in detail in the Reproduction module.

Neonatal gonorrhea occurs in a woman who gives vaginal birth to an infant while actively infected, resulting in **ophthalmia** and **blindness** if not treated (most common cause of neonatal blindness). Prophylactic erythromycin drops are given to all neonates.

Reactive arthritis, eponym Reiter's syndrome, is an arthralgia caused by a mucosal infection by *Neisseria gonorrhoeae*. The patient is generally not so toxic that they need an arthrocentesis (joint tap) to assess for infection. The bacteria is not in the joint; the pain an immune phenomenon.

Disseminated gonorrhea presents with arthralgias and **inflamed, actively infected joints**. Arthrocentesis reveals > 50,000 neutrophils, and you have to know to culture on a Thayer-Martin agar. The patient is toxic, has loss of function of the joint, and the bacteria are in the joint.

Diagnosis. If able to be seen, **intracellular** Gram-negative diplococci **within neutrophils** will appear on a smear. If an intracellular pathogen is seen within a neutrophil, it is gonorrhea. However, most smears are negative because the pathogen is hidden within the cell and escapes the stain. While Thayer-Martin agars are required for culture, a more rapid **nucleic acid amplification testing** (NAAT, PCR) on urine samples is often sufficient to make the diagnosis.

Treatment. Ceftriaxone is used. For cervicitis or urethritis, only a single intramuscular dose is needed. Because gonorrhea and chlamydia are so often found together, if you find gonorrhea, treat chlamydia with azithromycin (once) or doxycycline (for a week).

Moraxella catarrhalis ("The Other Other *Strep. Pneumoniae*")

This organism is a common cause of bronchitis and bronchopneumonia (in elderly patients with chronic pulmonary disease), sinusitis, and otitis. It is the **other other *Strep. pneumo***.

It **looks like a** *Neisseria* species, but **acts like** *Strep. pneumo* and *Haemophilus influenzae*.

Physiology/Structure. *Moraxella* looks much like *Neisseria* species in that it is a **Gram-negative diplococcus**. However, it is **not intracellular** and grows on **normal blood agar**. It is part of the normal flora of the upper respiratory tract and can be transmitted through respiratory droplets. The diseases it causes are the same as *Haemophilus influenzae*. *Strep. pneumo* is the most common cause of otitis media, sinusitis, and bronchitis/pneumonia. *Haemophilus influenza* is second. *Moraxella catarrhalis* is third. Good news is that empiric coverage for strep in any of these cases also doubles as treatment for *Moraxella*.

Haemophilus influenzae ("The Other *Strep. pneumo*")

Haemophilus influenzae does not cause the disease influenza (which is caused by a virus). *Haemophilus influenzae* is **the other *Strep. pneumo***. *Strep. pneumo* causes meningitis, otitis media, sinusitis, and pneumonia. So too, does *Haemophilus influenzae*. With the advent of a vaccine against the polysaccharide capsule, invasive disease has greatly diminished, though nonencapsulated forms have arisen, provoking milder infections. *Haemophilus influenzae* has another clinically relevant bacterium in its genus: *Haemophilus ducreyi*, which causes a painful chancre/ulcer of the genitals, and comes on the differential for genital lesions that cause pain (such as herpes simplex virus). We are not going to discuss *ducreyi* further, but will be sure to use the full genus-species combination *Haemophilus influenzae* to remind you that this discussion is not about the genus, but about this species. *Haemophilus influenzae* is commonly termed just "Haemophilus" in medical microbiology vernacular.

Physiology/Structure. *Haemophilus influenzae* is a small, pleomorphic Gram-negative coccobacillus that we are calling a coccus. To culture it, it must be grown on a **chocolate agar** with **factor X** (hemin) and **factor V** (NAD, vitamin factor). *Haemophilus influenzae* can grow in one other medium—a regular blood agar also growing *Staph. aureus*. This is called the **satellite phenomenon**, whereby the *Haemophilus influenzae* is fed factor X and factor V by the cytolytic properties of the staph colony releasing the factors from red blood cells.

Virulence. The major virulence factor in *Haemophilus influenzae* **type b** is the antiphagocytic polysaccharide capsule. That capsule is what allowed us to generate a vaccine. Since *Haemophilus influenzae* colonizes the mucosal epithelium, it is not surprising that it has **pili** for attachments and **IgA protease** to defend against mucosal antibodies.

Epidemiology. In unvaccinated populations (outside the United States), epiglottitis and meningitis are common diseases and commonly caused by Hib strains. In the US, where Hib has mostly been eradicated, the highest prevalence of *Haemophilus influenzae* is noncapsular species simply colonizing the respiratory epithelium or causing pneumonia. It is spread by aerosols and respiratory droplets. Hib is making a comeback in unvaccinated children and in the very elderly where vaccination wanes.

The other *Strep. pneumo*. *Strep. pneumo*, *Haemophilus influenzae*, and *Moraxella catarrhalis* are the most common causes of otitis media (ear), sinusitis (sinuses), and bacterial pneumonia (lungs). They colonize anyone and migrate to these regions. *Haemophilus influenzae* particularly likes chronic lung disease patients such as COPDers. Link COPD-pneumonia and *Haemophilus influenzae*.

Meningitis. *Haemophilus influenzae* type b **was** the most common cause of pediatric meningitis. Vaccination immunity has eliminated this presentation in the US. Disease in nonimmune patients results from bacteremic spread of the organisms from the nasopharynx and cannot be differentiated clinically from other causes of bacterial meningitis—fever, headache, stiff neck, photophobia. Unlike *Neisseria meningitidis*, which is rapidly progressive (which is the exception), *Haemophilus influenzae* and *Strep. pneumo* meningitis is subacute, over days. The initial presentation is a 1–3-day history of mild upper respiratory disease, after which the typical signs and symptoms of meningitis appear.

Epiglottitis. Epiglottitis is caused by the inflammation of the epiglottis, which **caused** a life-threatening narrowing of the airway. The epiglottis protects the larynx and trachea from food during swallowing, folding over the opening to the larynx, preventing food from going in. This also prevents air from going in. When it inflames, it gets large enough that it obscures the larynx at rest. Presenting with **stridor**, it warrants emergent airway control. The problem **was** that to pass an endotracheal tube into the larynx you would need to move the epiglottis. If you poke an inflamed epiglottis, it might get more inflamed and seal the airway. **Vaccines have nearly eliminated epiglottitis**, and thus the emphasis on the past tense.

Treatment. Amoxicillin/clavulanate (otitis and sinusitis, like *Strep. pneumo*), ceftriaxone + vancomycin + corticosteroids for meningitis (like *Strep. pneumo*).

Neisseria meningitidis	Gram-negative diplococci, spread by respiratory droplets Grows on Thayer-Martin agar (chocolate agar with abx), **ferments maltose** **Capsule** (vaccine), IgA protease, pili, lipo-oligo-saccharide endotoxin Rapidly progressive meningitis, **petechial rash, adrenal insufficiency**, **DIC** Treatment: Ceftriaxone, vancomycin, prednisone; Prophylaxis: Rifampin **Vaccine** available: college/military
Neisseria gonorrhoeae	Gram-negative diplococci, **sexually transmitted infection** **Facultatively intracellular, found inside neutrophils** Grows on Thayer-Martin agar (chocolate agar with abx) No capsule (no vaccine possible), pili and opa adhere, porins exchange nutrients Male urogenital infection—purulent penile discharge Female urogenital infection—asymptomatic, **cervicitis** (painful sex, vaginal discharge), **acute pelvic inflammatory disease** (ascending infection), Fitz-Hugh–Curtis Reactive arthritis—arthralgias in joints because of gonorrhea in mucosa **Disseminated arthritis**—arthritis in joints because gonorrhea **in joints** Treatment: **Ceftriaxone** for gonorrhea, azithro or doxy for chlamydia always included Neonatal blindness—erythromycin drops prophylaxis
Haemophilus influenzae	Gram-negative coccobacillus, spread by respiratory droplets/aerosols Grows on chocolate agar only with **factor X** (hemin) and **factor V** (NAD, "vitamin") Hib (type b) vaccine prevents encapsulated form causing **meningitis**, **epiglottitis** Nonencapsulated infections cause otitis media, sinusitis, and pneumonia (**the other *Strep. pneumo***), especially in smokers Treatment: Amoxicillin/clavulanate
Moraxella catarrhalis	Gram-negative diplococci, spread by respiratory droplets Grows on normal blood agar Causes otitis media, sinusitis, and bronchitis (**the other other *Strep. pneumo***) Treatment: Amoxicillin

Table 7.2: Summary Table